bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Microscopy and live-cell imaging

PU Perunthottathu Umasankar

Updated date: May 12, 2021

An abbreviated version of this protocol was published in eLIFE in Oct 2014

A clathrin coat assembly role for the muniscin protein central linker revealed by TALEN-mediated gene editing

DOI: 10.7554/eLife.04137

Detailed protocol

Microscopy and live-cell imaging

Microscopy

1. Image fixed and fluorescently stained samples on an inverted Olympus Fluoview FV1000 confocal microscope.
2. Use PlanApo N (60 × /1.42 numerical aperture) oil objective for imaging.
3. Acquire Z-stacks of cells with 0.25-µm step size between optical sections using appropriate lasers and FV10-ASW software.
4. Deconvolve stacks using blind deconvolution algorithm in Autoquant X3 to remove noise.

Live-cell imaging

1. Maintain cells in DMEM supplemented with 10% fetal calf serum and 25 mM HEPES, pH 7.2 on glass bottomed MatTek dishes in a CO_2 controlled humidified chamber at 37°C.
2. Perform live imaging using total internal reflection fluorescence (TIRF) optics at 37°C with a Nikon Ti microscope equipped with both confocal (A1R spectral), wide field, and through-the objective type TIRFM capabilities.
3. Use 60 × 1.49 NA oil-immersion objective for imaging.
4. Focus the cells using drift correction device and lock the Z-position.
5. Capture images with Andor Zyla 5.5 camera at full frame with no binning and with a 4 color (405 nm, 488 nm, 561 nm and 647 nm lines) laser launch.
6. Select individual fields blindly and collect sequential images using conditions that caused minimal signal loss due to bleaching with the illuminating laser.
7. Laser conditions vary with experiments, either YFP alone (488 nm excitation 525/40 nm emission filter) or with the 561 nm (600/50 emission filter) or 647 nm (700/75 emission filter).
8. To ensure perfect image registration between colors, use a single 4 color TIRFM filter cube with a high speed (20 ms change time) filter wheel to block bleed through between channels.
9. Acquire images continuously at 5s/ frame using NIS Elements software.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Umasankar, P. (2021). Microscopy and live-cell imaging. Bio-protocol Preprint. bio-protocol.org/prep1082.
2. Umasankar, P. K., Ma, L., Thieman, J. R., Jha, A., Doray, B., Watkins, S. C. and Traub, L. M.(2014). A clathrin coat assembly role for the muniscin protein central linker revealed by TALEN-mediated gene editing. eLIFE. DOI: 10.7554/eLife.04137